Evaluation of a promising new homogenous assay technology (SPARCL) and comparisons with MSD, using FSH as test substance

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Background

Bioanalysis department at Ferring

Small team working with LBA assays (Six people of which three in the lab)

Now

Studies: Main focus on TK, PK, ADA, and NAb analysis. Biomarker studies are mostly outsourced

Assay technology: MSD and ELISA

The future

Studies: More biomarker – and early development studies will be run in-house

Challange: **Fast assay development time** to test early project ideas

Assay technology: MSD, ELISA, additional technology with shorter assay time – **SPARCL?**

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FSH (Follicle Stimulating Hormone)

- Ferring is a world leader in Assisted Reproductive Technology (ART) for fertility treatment
- FSH-based products on the market: MENOPUR® and BRAVELLE®
- Several analytical methods developed for different FSH formulations over the years (mostly on the MSD instrument) → good basis for comparison with SPARCL
- Reference method:
  - **LLOQ:** 0.075 ng/mL
  - **ULQ:** 5.00 ng/mL
  - **Assay range:** 0.035-5.00 ng/mL (8 CAL levels)
  - **Assay time:** 5 hours (excluding overnight incubation of capture antibody)
  - **MRD:** 1:6
  - **Assay design:** Sandwich ELISA (antibodies directed against α- and β-subunit of molecule respectively)
  - **Matrix:** Human serum (low endogenous FSH levels)

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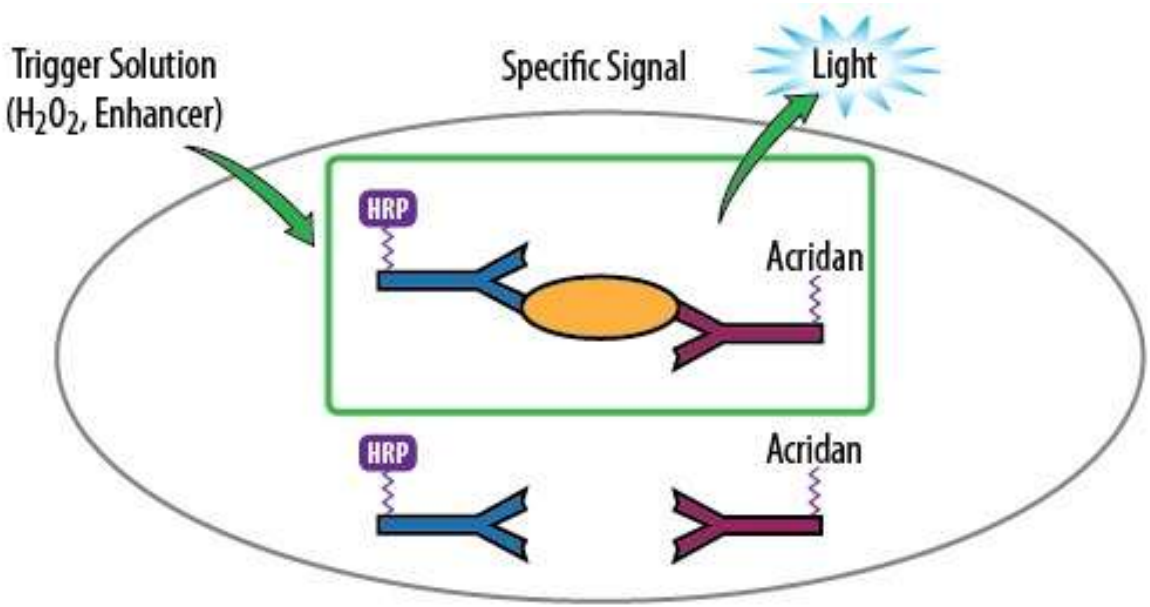
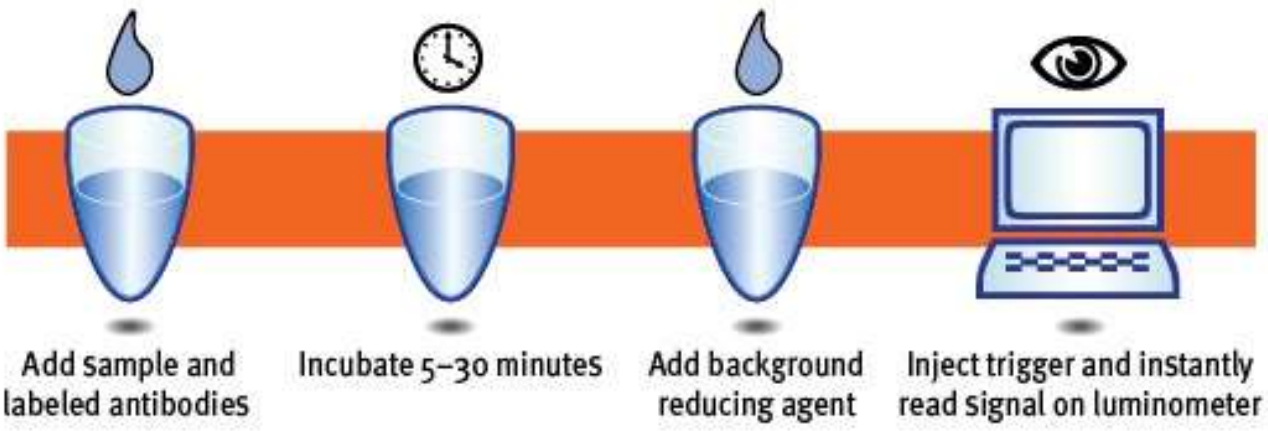
SPARCL (Spatial Proximity Analyte Reagent Capture Luminescence)

- Developed by Lumigen (A Beckman Coulter Company)
- Proximity dependent chemiluminescence technology (flash luminescence)
- Homogenous assay format (no wash steps)
- Kit-based – no unique instrumentation



Simple and fast assay protocol



Instrument and reagents

Part of the of the SPARCL-kit (SDK-1K kit) – *enough for 8-10 plates*

- One vial acridan-label (substrate to HRP that gives off light)
- 100 mL Trigger solution (hydrogen peroxide based, triggers the luminescent reaction)
- 10 mL Background reducing reagent (BGR) (anti-oxidant, added just prior to analysis)

Not SPARCL-specific components

- Luminometer with injector that enables instant read-out
  (e.g. SpektraMax-L, LUMIStar Omega)
- White microplates for luminescent assays
- HRP-labelled reagent (easy to label yourself or
  often commercially available in conjugated form)

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Potential advantages

- Reduced assay time
- No need for unique instrumentation
- Gyros and MSD like performance without the capital expense
- No expensive discs or plates
- Simple and straight forward assay protocol
- All binding reactions in solution offers improved kinetics
- LIMS compatible
- Immediate generation of the flash signal upon triggering reduces reading time
- Automation friendly
- Rapid immunoassay development
  - Assay qualification/validation within a week
  - Several assay development runs per day (30-60 minute assay length)
- Assays with wide dynamic ranges
- Sensitive assays

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Evaluation

Analyte: Recombinant FSH (WHO standard 92/510) – *no Ferring product*

Instruments: SpectraMax-L (Molecular Devices) & LumiSTAR Omega (BMG)

Plates: White Greiner F-bottom microplates

Optimized Working Antibody Concentrations
- 25 µL HRP-mxFSH-a (1:1000, ~0.83 mg/mL)
- 25 µL Acridan-mxFSH-b2 (1:100, 2.5 mg/mL)
- 50 µL Sample (neat serum – final MRD 1:4)

} 1 hour incubation time

BGR Volume – added just before plate is injected to instrument
- 5 µL

Trigger volume – added to each well just before read-out
- 100 µL

Assay range:
- 0.035-5 ng/mL (8 CAL levels)

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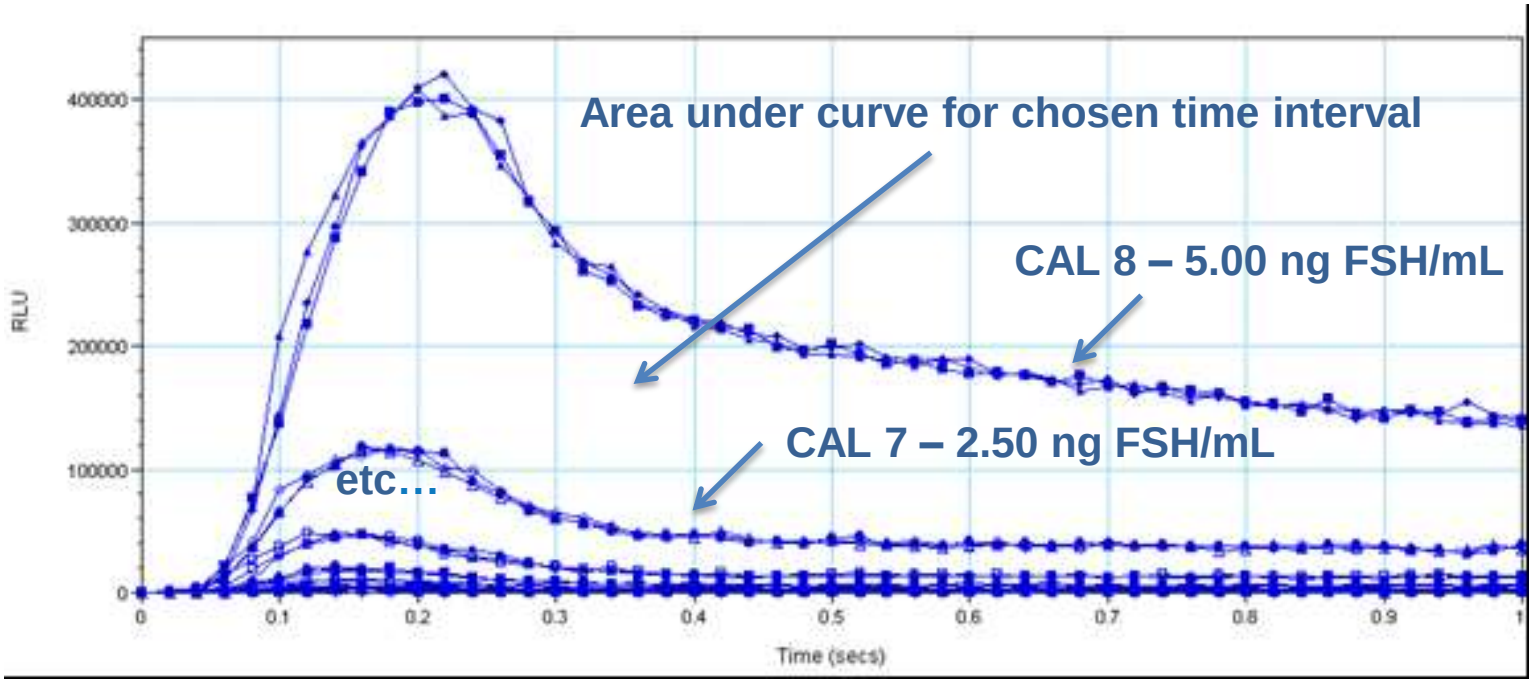
Evaluation

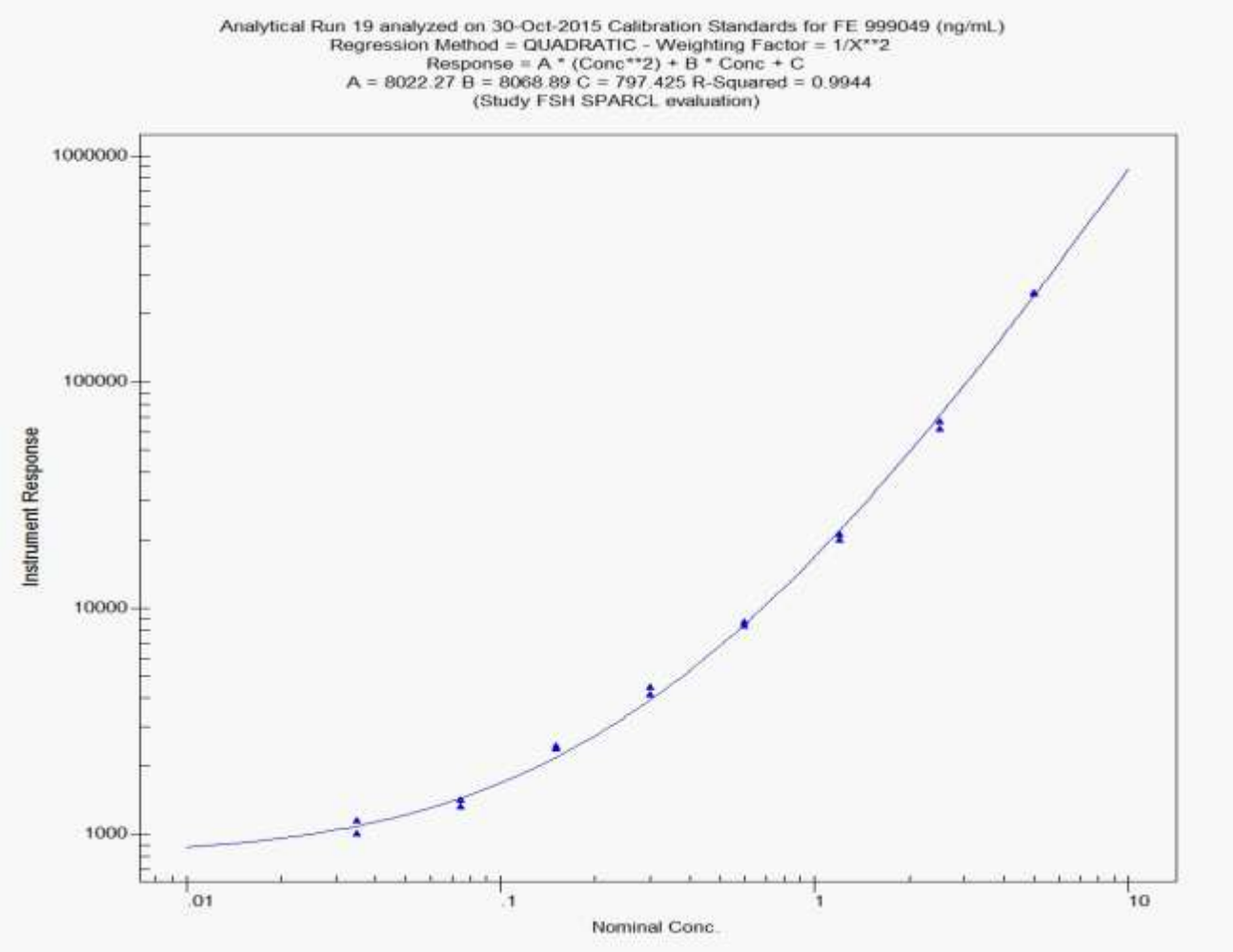
Flash luminescence curves from different calibration samples on SpectraMAX-L



- 50 measurement points per second
- 1 s reading time/well

Evaluation

Example of calibration curve



Evaluation

Precision and Signal to blank ratio of calibration samples

FSH (ng/mL)	Rep1	Rep2	Rep3	Mean	SD	%CV	S:B ratio
0	865	924	886	891	29.7	3	N/A
0.035	1130	1250	1198	1192	60.2	5	1.34
0.075	1435	1342	1554	1443	106	7	1.62
0.15	2379	2318	2234	2310	72.8	3	2.59
0.3	3960	4036	3789	3928	127	3	4.41
0.6	7628	7374	7410	7470	137	2	8.38
1.2	17474	18616	17287	17792	719	4	20
2.5	48400	49923	48921	49081	774	2	55.1
5	196649	199706	199967	198774	1845	1	223

- Good precision across analytical range
- Low S:B ratio acceptable due to consistent blank signal

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Evaluation

Precision & accuracy – QC samples

	LOW	MID	HIGH
FSH (ng/mL)	**0.225**	**0.700**	**3.75**
	0.238	0.674	2.68
	0.235	0.741	2.75
	0.238	0.611	2.60
	0.229	0.593	2.67
Average:	0.235	0.655	2.68
SD:	0.004	0.07	0.06
CV (%):	**2**	**10**	**2**
Mean bias (%):	**4**	**-6**	**-29**
n	4	4	4

Preparation error?

- Good precision & accuracy for all levels **except QC HIGH** – preparation error suspected

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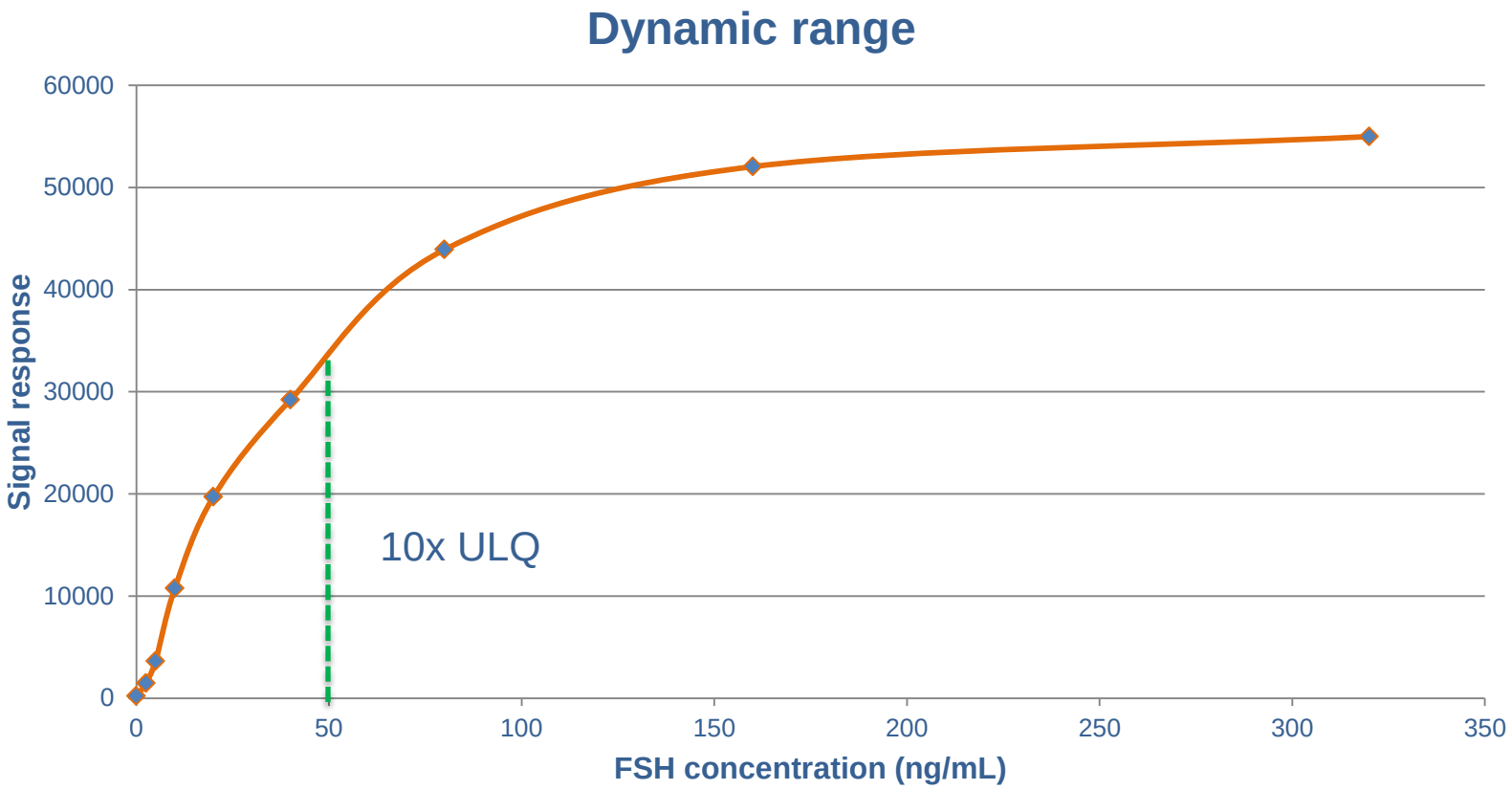
Evaluation



- Wide dynamic range well above the chosen ULQ level
- No Hook effects

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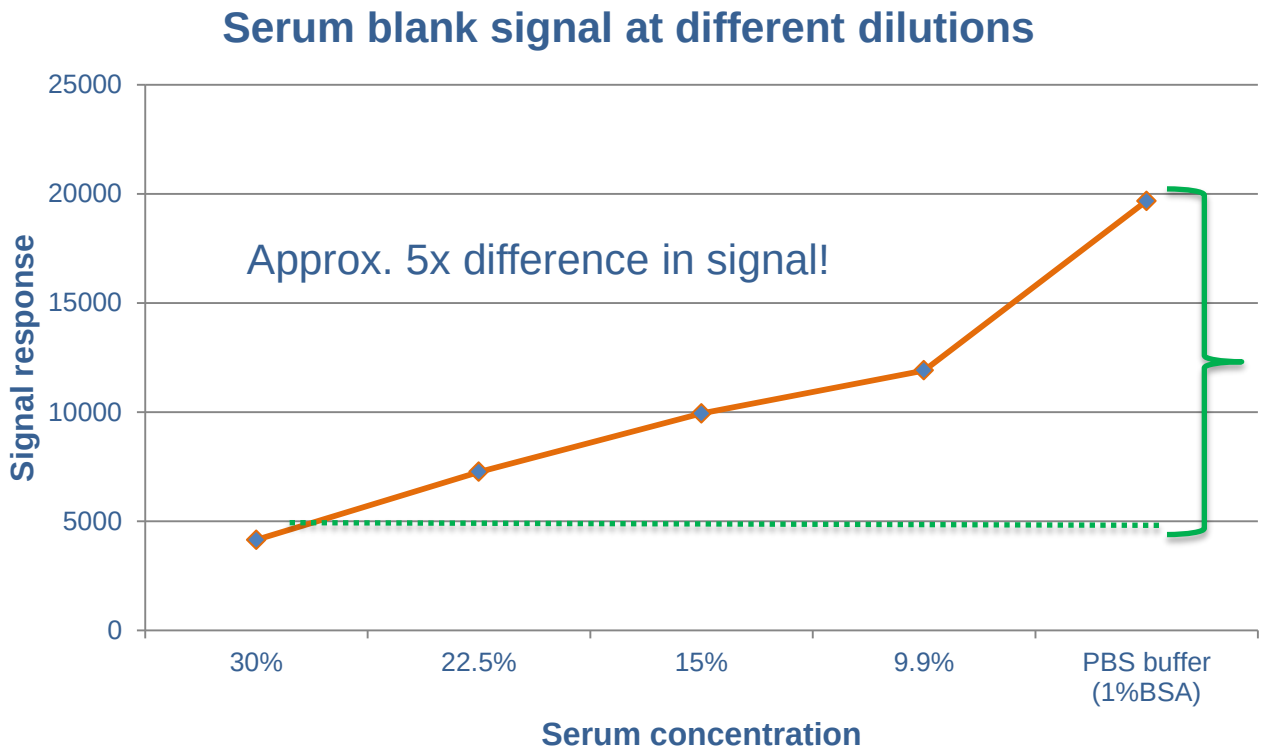
Evaluation



- Dilution of sample in PBS buffer (with 1%BSA) increased blank signal!
- Protein has suppressing effect on the SPARCL signal – use higher protein content in buffer (e.g. 10%BSA) in order to mimic the protein content in neat serum

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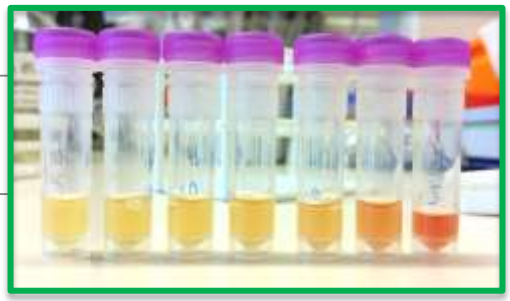
Evaluation



Hemolysis
Signal response
30000
25000
20000
15000
10000
5000
0
HIGH (reference)
HIGH (hemolysis 0.1%)
HIGH (hemolysis 0.2%)
HIGH (hemolysis 0.3%)
HIGH (hemolysis 0.4%)
HIGH (hemolysis 0.5%)
HIGH (hemolysis 1%)
no pre-dilution
2x pre-dilution
4x pre-dilution
20x pre-dilution

Conclusions

Advantages

- Assay transfer fast and easy
  - *Less than one week*
- Precision/accuarcy
- Dynamic range
- Costs
- Assay time

Drawbacks

- Prominent matrix effects
  - *hemolysis, signal suppression*
- Sensitivity
  - *High MRDs required in ”problematic” matrices which diminishes sensitivity*

Potential applications at Ferring

- TK-studies
- Early drug development
- Biomarkers

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